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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

WANG et al.

Serial No.: 09/636,243 Art Unit: 1645

Filing Date: August 10, 2000 Examiner: Unassigned

Title: DIMERIZING PEPTIDES

PRELIMINARY AMENDMENT AND SUBMISSION OF SUBSTITUTE SPECIFICATION PURSUANT TO 37 C.F.R. 1.125

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This preliminary amendment is submitted concurrently with a substitute specification filed pursuant to 37 C.F.R. 1.125. Examination of the substitute specification is respectfully requested.

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I. AMENDMENTS

Please substitute the specification attached hereto. The substitute specification incorporates the following amendments:

Inserted. on the original spec

In the Cross-Reference to Related Applications:

On page 1, line 5 of the original specification "August 11, 2000" has been replaced by --August 11, 1999--.

In the Brief Description of the Drawings:

On page 5, line 3, the following information has been inserted:

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Fig. 3 (A) Sketch showing key segments of the phagemid. (B) Expected arrangement of fusion proteins at the target DNA. Phage displaying two copies of a dimerizing peptide-Zif12 fusion can form stable complexes with the biotinylated target DNA site, which contains an inverted repeat of the Zif12-binding site. The phage-DNA complexes are captured by streptavidin coupled to a solid support, and phage that bind less tightly are washed away. (C) The DNA site used for affinity selection of phage, with the two juxtaposed Zif12-binding sites in bold.

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Fig. 4 (A) Sequences of peptide extensions isolated from the initial selection. Numbers in parentheses give the frequency of occurrences among the 45 clones sequenced. The clones for peptide 2 included a Glu-21-to-Asp mutation in the zinc finger region that may have been partially responsible for the affinity of this peptide. (B) Gel mobility-shift assays using purified fusion peptides 1, 3, and 5. Protein (2.5 μM, 250 nM, 25 nM, 250 pM, and no protein) was incubated with DNA containing either an inverted repeat of Zif12 sites or a single Zif12 site and then electrophoresed through native polyacrylamide gels. The reduced mobility of the inverted repeat probe in the

presence of protein indicates the formation of protein-DNA complexes. Similar results were obtained with fusion peptides 2 and 6. Binding of peptide 4 depend on disulfide bond formation.

Fig. 5. Overall scheme for sequential reoptimization of peptides. A 15-residue peptide obtained from the initial selection was divided conceptually into three blocks of 5 amino acids (aa) each and reoptimized in three steps. In the first step, the five-residue block closest to the fingers was completely randomized, with the other 10 aa held constant. Phage display of the new fusion proteins, with six to nine selection and amplification cycles, was used to obtain the best sequences from this pool. In the second reoptimization step, the central five-residue block was completely randomized, with the finger-proximal region held constant as the newly optimized sequence and the finger-distal region corresponding to the initially selected sequence. The best sequences from this pool were obtained again via phage display with a series of selection and amplification cycles. In the final reoptimization step, the finger-distal five-residue block was completely randomized and then reselected in the context of the two other reoptimized blocks.

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Fig. 6. Evolution of peptides 1 and 5 by sequential block reoptimization. The sequences selected from each reoptimization step are shown in bold, with the number of selection and amplification cycles given in parentheses. Sequences roughly matching the consensus that were used in later steps have been boxed. In some cases, such as in reoptimization step 3 for peptide 1 and reoptimization step 1 for peptide 5, we isolated clones that carried spurious mutations at a nondegenerate position of the peptide extension. In addition, the E21D mutation in the zinc finger region (which also was seen in the original peptide 2 sequence) arose several times; this mutation may stabilize complex formation by improving contacts at the protein-DNA interface. Note: some confusion was caused by this E21D mutation, which occurred in the first reoptimization step for peptide 1, but was discovered only after reoptimization step 2. Thus, the "consensus" sequence from reoptimization step 1 (VPKQR), chosen after selection-

amplification cycle 9, had a glutamine that did not occur in sequences isolated after cycle 6. To double-check this position of peptide 1, it was randomized again during reoptimization step 3. The corresponding position was allowed to vary as Q, M, I, or L, along with the complete randomization of the third block. The reselections showed that methionine or leucine is preferred at this position.

Fig. 7: Comparison of the contact surfaces of various zinc fingers. Zif268 finger 1, GLI finger 1, GLI finger 2, and SW15 finger 1 are shown. (A) Stereoview of a superposition of the four fingers . Because of the different lengths of the fingers, the superposition aligned $C\alpha$ atoms of residues 104-114 and 116-127 of Zif finger 1 with those of residues 3-13 and 15-26 of GLI finger 1; residues 105-114 and 116-130 of Zif finger 1 with 37-46 and 51-65 of GLI finger 2; and residues 104-130 of Zif finger 1 with 31-57 of SWI5 finger 1. Side chains on each finger that are involved in hydrophobic contacts at the corresponding protein-protein interface have been rendered in stick representation. (Coordinates for GLI and SW15 are from Pavletich *Science* **261**, 1701 (1993); Dutnall, *Structure* **4**, 599 (1996).) (B) Alignment of the sequences of the fingers, with interacting residues boxed. The portion of Zif268 shown here corresponds to residues 104 to 130 in the crystal structure.

Fig 8. Crystal structure of our zinc finger homodimer and comparisons with the MATa1/α2 homeodomain heterodimer. (A) Overview of our homodimer complex. The protein monomers bind in a head-to-head orientation on the DNA; the peptide extension and zinc fingers for the monomers are labeled. The complex is approximately symmetric with a twofold axis that goes through the center of the DNA and is perpendicular to the plane of the page. There are two zinc fingers in each monomer, and these bind essentially as observed in the wild-type Zif268 complex (finger 2 for each monomer is hard to see in this figure since it is almost directly behind finger 1 in this view of the complex). (B) Crystal structure of the MATa1/α2 heterodimer-DNA complex as determined by Wolberger and colleagues, *Ann Rev Biophys. Biomol. Struct.*

28, 29 (1999); Li et al., Science 270, 262 (1995). A peptide extension from the MATα2 homeodomain contacts an exposed hydrophobic surface on the MATa1 homeodomain to facilitate cooperative binding.

Fig 9. Peptide-protein contacts at the dimer interface of our zinc finger complex. For simplicity, only the contacts in one half of the symmetric dimer are shown; an equivalent set of contacts is seen in the other half of the dimer. (A) View of the dimer interface showing the peptide extension fitting against the zinc finger (surface representation). (B) Diagram highlighting key residues of the peptide extension and zinc finger 1 at the dimer interface.--

In the Detailed Description:

On original page 12, line 1 "I" has been replaced by --II--.

On original page 17, line 9, "II" has been replaced by --III--.

On original page 19, line 1, "III" has been replaced by --IV--.

On original page 20, line 23, "III" has been replaced by --V--.

On original page 24, line 1, "IV" has been replaced by --VI--.

Original pages 27 through page 32 (Example 1) have been inserted as text and extraneous headings have been removed, as indicated in red ink. Furthermore, the Figures embedded in these pages are now described in the "Brief Description of the Drawings" as Figures 3 through 6 and, accordingly, references to these Figures in Example 1 now reflect the proper Figure designation.

Original pages 33 through 49 (Example 2) have been inserted as text. Extraneous headings have been removed. The footnotes on original pages 40-44 have been inserted into the text at the appropriate locations. The Figures on original pages 45 to 47 have been described in the "Brief Description of the Drawings." In particular, original Figure